ORIGINAL ARTICLE

Increased Resting and Exercise-Induced Oxidative Stress in Young IDDM Men

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OBJECTIVE — To assess the effect of acute physical exercise on oxidative stress and glutathione redox status and the relation to physical fitness in otherwise healthy young men with IDDM.

RESEARCH DESIGN AND METHODS — Nine men with IDDM (HbA_{1c} 7.3 ± 1.7%), ages 21–30 years, and 13 matched control subjects exercised on a bicycle ergometer for 40 min at 60% of their maximal oxygen consumption (Vo_{2max}). Oxidative stress was assessed with plasma thiobarbituric acid reactive substance (TBARS) levels (an index of lipid peroxidation) and, in response to exercise, also glutathione redox status. For glutathione redox status, blood total glutathione (TGSH) and oxidized glutathione (GSSG) were determined. Blood samples were drawn immediately before and after exercise.

RESULTS — Resting plasma TBARS levels were markedly elevated in diabetic patients (2.2 ± 0.7 vs. 0.9 ± 0.4 μmol/l; $P = 0.0002$). Mean blood TGSH was higher in diabetic subjects (1,203 ± 221 vs. 936 ± 156 mmol/l; $P = 0.002$), with no significant difference in GSSG or GSSG/TGSH values. Exercise increased plasma TBARS and blood GSSG by ~50% in both groups. Resting plasma TBARS had a strong inverse correlation ($r = -0.82$; $P = 0.006$), and the exercise-induced percentage increase in TBARS had a strong positive correlation ($r = 0.81$, $P = 0.008$) with Vo_{2max} in diabetic subjects only.

CONCLUSIONS — Glutathione redox status appears to be adequate in healthy young moderately active diabetic men. On the other hand, they demonstrated increased resting and postexercise oxidative stress as indicated by plasma TBARS. Although exercise acutely induces oxidative stress, in patients with diabetes, physical fitness may have a protective effect against oxidative stress.

Oxidative stress has been increasingly implicated in atherosclerosis (1–3) and in the accelerated atherosclerosis and microvascular complications of diabetes (2,4–5). Oxidative stress can result in widespread lipid, protein, and DNA damage (6), including oxidative modification of LDL cholesterol, believed to be central in the pathogenesis of atherosclerosis and endothelial dysfunction (1–3,5). Oxidative stress as assessed by indexes of lipid peroxidation has been shown to be elevated in diabetes (7–14), even in patients without complications (9,11–13). The mechanisms underlying the apparent increased oxidative stress in diabetes are not entirely clear. Accumulating evidence points to many, often interrelated mechanisms (2,4–5), increasing production of free radicals (15) or decreasing antioxidant defenses (16). These mechanisms include glucose autoxidation (17) and formation of advanced glycosylation end products (AGEs) (2,18), activation of the polyol pathway (4), and altered cell and glutathione redox status (19) and ascorbate metabolism (20), and perturbations in nitric oxide and prostaglandin metabolism (5).

Most studies have found decreased total glutathione (TGSH) levels or increased oxidized glutathione (GSSG) to TGSH ratios in diabetes (19,21–24). Reduced glutathione (GSH) is a ubiquitous endogenous thiol with well established antioxidant properties (25). GSH and its associated enzymes form one of the key antioxidant enzyme systems in the cytosol. When exposed to free radicals, GSH is oxidized either spontaneously or via glutathione peroxidase to form GSSG. NADPH-dependent glutathione reductase, in turn, reduces GSSG to GSH. The relative quantities of blood GSSG and TGSH are thus often used as a reflection of erythrocyte glutathione redox status (19,21,25).

Exercise has been widely recommended for IDDM patients (26). The potential benefits and risks of exercise may be particularly important for diabetic patients, who at rest already show higher levels of oxidative stress. Many recent studies have established that even moderate exercise increases free radical production beyond the capacity of antioxidant defenses, resulting in oxidative stress (27–30). Regular exercise can strengthen antioxidant defenses and may reduce resting and acute exercise-induced oxidative stress (29–30). To our knowledge, no studies have been published regarding acute exercise-induced oxidative stress in diabetes.

The purpose of this study is to assess 1) oxidative stress at rest as measured by a widely used index of lipid peroxidation; 2) glutathione redox status; 3) the effect of acute sustained exercise of moderate intensity on indexes of oxidative stress; and 4) the relation of resting and exercise-induced oxidative stress to fitness in healthy young men with IDDM in moderate glycemic control.

RESEARCH DESIGN AND METHODS —

Young otherwise healthy men with IDDM

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AGE, advanced glycosylation end product; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substance; TGSH, total glutathione.

Table 1—*Characteristics of diabetic and control subjects*

	Diabetic group	Control group
n	9	13
Age (years)	23 ± 1.7	23 ± 2.9
BMI	23.5 ± 2.5	23.3 ± 1.7
Vo_{2max} (ml · kg^{-1} · min^{-1})	46 ± 6.9	45 ± 6.0
Exercise frequency (times/week)	2.6 ± 1.6	1.2 ± 1.6
Duration of diabetes (years)	9 ± 5.8	N/A
HbA_{1c} (%)	7.3 ± 1.7	N/A
Daily insulin dose (IU/kg)	0.6 ± 0.3	N/A
Serum cholesterol (mmol/l)	4.3 ± 0.5	4.9 ± 1.0
HDL (mmol/l)	1.2 ± 0.3	1.5 ± 0.5
Serum triglycerides (mmol/l)	1.5 ± 1.2	1.1 ± 0.4

Data are means (SD).

($n = 9$) and healthy male control subjects ($n = 13$) were recruited into the study after obtaining informed consent. Diabetic subjects were chosen from patients followed at the Kuopio University Hospital Diabetes Clinic. Control subjects were volunteers from the local university student population. Clinical and biochemical data are displayed in Table 1. Diabetic and control subjects were well matched in terms of age, BMI, and physical fitness (as measured by Vo_{2max}, an accepted index of cardiorespiratory fitness) (31). All participants underwent clinical examination, routine laboratory tests, and electrocardiograms to rule out significant diseases. Reasons for exclusion included any cardiovascular or pulmonary disease, vitamin supplementation, chronic medication other than insulin, and regular participation in organized athletic events or highly intense physical activity, arbitrarily defined as >5 h a week of physical exercise causing breathlessness or raising a sweat. Reported physical activity tended to be higher ($P = 0.06$) in the diabetic group than in the control group (Table 1). None of the diabetic subjects had clinically evident atherosclerotic disease, nephropathy (overnight urinary albumin excretion was <10 μg/min and serum creatinine was normal), or neuropathy, and only two of them had mild background retinopathy, not requiring laser treatment. Diabetic subjects were in fair glycemic control (the mean HbA_{1c} of the last two measurements over the preceding 6–8 months was 7.3 ± 1.7%).

Exercise testing

All subjects underwent a maximal exercise test to determine Vo_{2max}, using an electrically braked bicycle ergometer, breath by breath gas monitoring, and continuous electrocardiogram monitoring to determine the Vo_{2max} for each subject. Testing began at 60 W and was increased by 30 W every 2 min. Maximal effort was defined subjectively by the subjects' maximal voluntary effort or objectively (oxygen consumption increase of <150 ml/min despite increasing workload).

One to two weeks later, subjects exercised for 40 min at 60% of their Vo_{2max} after 5 min warm up at 60 W. Before exercising, all subjects refrained from intense exercise and alcohol consumption for at least 3 days before exercise testing, and from smoking for at least 24 h for the one smoker in each group. On the day of exercise, the subjects ate a light carbohydrate rich breakfast. Diabetic subjects decreased their usual rapid acting insulin dose as appropriate. Exercise tests were carried out 2–4 h after breakfast. The study was approved by the Ethics Committee of the University of Kuopio.

Blood sample collection and preparation

Routine screening laboratory tests, lipoprotein profiles, and HbA_{1c} analyses were determined by blood samples drawn in a fasting state in the morning on a separate day from the exercise tests. Samples for blood glutathione and plasma TBARS assays were taken from an antecubital vein 5 min before and within 2 min after exercise at 60% Vo_{2max} for 40 min. Fingerstick blood glucose determinations were done 10 min before and within 5 min after exercise at 60% Vo_{2max} in the diabetic group only.

Blood TGSH and GSSG analyses were done as described before (32). Briefly, for TGSH determinations, EDTA blood was precipitated with perchloric acid and the deproteinized supernatant was used. For blood GSSG, the clear supernatant obtained from EDTA blood treated with 5-sulfosalicylic acid was neutralized and reacted with 2-vinylpyridine. Treated samples were frozen at −20°C until spectrophotometric determination.

Plasma TBARS was assayed as described previously (33). Briefly, for the determination of TBARS, EDTA blood was added to CHELEX (Bio-Rad, Richmond, CA) treated potassium phosphate buffer containing Na_2EDTA immediately after being drawn. The mixture was briefly centrifuged to obtain plasma. The plasma was immediately treated with ethanolic butylated hydroxytoluene. Treated plasma was added to 25% trichloroacetic acid to precipitate plasma proteins. The mixture was then placed in a boiling water bath for 30 min to release protein-bound malondialdehyde (MDA). The tubes were centrifuged to obtain a clear supernatant. The supernatant was then frozen at −70°C until TBARS could be determined as described previously (33).

Serum cholesterol and triglyceride were measured enzymatically using a Hitachi 717 analyzer (Tokyo, Japan). The same method was also used for HDL after removal of LDL and VLDL by dextran sulphate/$MgCl_2$ (34).

Blood HbA_{1c} was measured in diabetic subjects using liquid cation exchange chromatography (normal range 4.0–6.0%) (35).

Data analysis

The SPSS/PC+ software (SPSS, Chicago, IL) was used for statistical analyses. Results for the groups are expressed as means ± SD. Differences between the group means were analyzed for significance using the unpaired Student's t test or the Mann-Whitney U test as appropriate. Differences within the same group before and after exercise were tested with analysis of variance repeated measures. Pearson's correlation and partial correlation analysis was used to assess the associations between selected variables. Statistical significance was defined as $P <$ 0.05.

RESULTS

Maximal oxygen consumption

The mean Vo_{2max} in both groups was similar (Table 1).

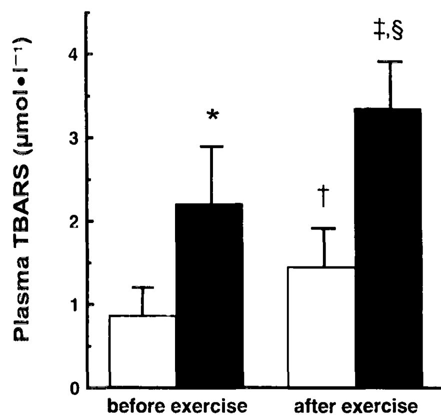

Figure 1—*Mean levels of plasma TBARS before and after exercise for the diabetic (■) and control (□) groups. Error bars represent SD. *P < 0.005, diabetic vs. control group, Mann-Whitney U test. †P = 0.001; diabetic group, before vs. after exercise. ‡P = 0.012; control group, before vs. after exercise. §P = 0.001, diabetic group vs. control group, Mann-Whitney U test.*

Oxidative stress and antioxidant indexes at rest

At rest, the diabetic group had a greater than twofold higher mean plasma TBARS (P < 0.0002; Fig. 1). Mean blood TGSH was ~30% higher in diabetic subjects (P < 0.002; Table 2). Mean blood GSSG was not statistically different in the diabetic group compared with the control group. The mean resting GSSG to TGSH ratio was also similar in both groups.

Response of oxidative stress and antioxidant indexes to sustained submaximal exercise

Plasma TBARS levels immediately after a 5 min warm up and 40 min of exercise at 60% of Vo_{2max} postexercise are depicted in Fig. 1. Mean plasma TBARS increased significantly by ~50% in both groups (diabetic group, P = 0.001; control group, P = 0.012). Mean blood TGSH did not change with exercise (Table 2). Mean GSSG, however, increased by at least 50% in both groups (diabetic group, P = 0.004; control group, P = 0.002). The GSSG to TGSH ratio showed a similar response to exercise in both groups (diabetic group, P = 0.007; control group, P = 0.002).

Fingerstick blood glucose levels in the diabetic group before and after exercise

Pre- and postexercise blood glucose levels were 10 ± 5 and 8 ± 5 mmol/l, respectively.

Correlations of Vo_{2max} and resting and acute exercise-induced lipid peroxidation

A strong inverse correlation between resting plasma TBARS and Vo_{2max} was found in the diabetic group (r = −0.82, P = 0.006; Fig. 2*A*). Adjustment for triglyceride levels at rest (r = −0.82, P = 0.024) or HbA_{1c} (r = −0.76, P = 0.027) using partial correlation analysis had only minimal effects on the correlation. Postexercise plasma TBARS did not correlate with Vo_{2max} (Fig. 2*B*). A strong positive correlation between the relative plasma TBARS increase (the ratio of postexercise TBARS to pre-exercise TBARS) in response to exercise and oxygen consumption during exercise (60% Vo_{2max}) was found in the diabetic subjects (r = 0.81, P = 0.008; Fig. 2*C*). Adjustment for triglyceride levels or HbA_{1c} had little effect on the correlation. These correlations were not present in the control group.

Other correlations

Resting plasma TBARS did not correlate significantly with HbA_{1c}, prevailing blood glucose levels, or duration of diabetes in diabetic subjects or with blood glutathione or lipid indexes in either group. HbA_{1c} did not correlate significantly with blood TGSH (r = −0.58, P = 0.10), Vo_{2max}, or fasting triglycerides.

Table 2—*Blood glutathione indexes before and after sustained submaximal exercise in diabetic and control groups*

	Before exercise		After exercise	
	Diabetic group	Control group	Diabetic group	Control group
n	9	13	9	13
TGSH (mmol/l)	1,203 ± 221*	936 ± 151	1,220 ± 249†	916 ± 116
GSSG (mmol/l)	108 ± 45	80 ± 47	173 ± 48‡	122 ± 58‡
GSSG/TGSH (× 1,000)	3 ± 37	84 ± 42	144 ± 74‡	135 ± 68§

Data are means ± SD. *P = 0.002, unpaired Student's t test, diabetic vs. control group. †P = 0.001, Mann Whitney U test, diabetic vs. control group. ‡P < 0.005, before vs. after exercise. §P = 0.007, before vs. after exercise.

CONCLUSIONS— A greater than twofold higher level of plasma TBARS, a widely used indirect measure of lipid peroxidation (36), was found in healthy young IDDM men both at rest and after exercise, suggesting increased oxidative stress. The most striking findings are the strong inverse correlation between resting plasma TBARS and Vo_{2max} and the strong positive correlation between the relative increase in plasma TBARS and Vo_{2max}, present only in the diabetic group and apparently independent of glycemic control or lipid levels at rest. The strong inverse correlation between resting plasma TBARS and Vo_{2max} suggests that physical fitness may have a protective role in minimizing elevated resting oxidative stress in diabetes.

In the TBARS assay, thiobarbituric acid is reacted with MDA, a product of lipid peroxidation, to form TBARS, which can be measured spectrophotometrically. Although not very specific (36), in vitro TBARS has been shown to be closely associated with hydroperoxide and plasma TBARS to be closely correlated (r = 0.73) with LDL susceptibility to oxidation (37). Vitreal TBARS and high-performance liquid chromatography–measured MDA have been found to correlate highly (r = 0.94) in diabetic patients (38). In a particularly insightful series of experiments, AGE-modified albumin increased TBARS in endothelial cells in vitro, and when injected into rats, AGE-modified albumin was found to increase levels of tissue TBARS, heme oxygenase, nuclear factor κB, and vessel wall MDA stained immunohistochemically (18). TBARS induction by AGE albumin in both in vitro and animal experiments could be blocked by probucol or *N*-acetylcysteine, well characterized antioxidants (18,33). These findings suggest that when interpreted cautiously, TBARS can be valuable as an index of oxidative stress.

The more than twofold higher level of resting plasma TBARS in the diabetic group suggests increased oxidative stress in diabetes and agrees with a large number of studies finding increased plasma TBARS or MDA in diabetic patients (7–14). The diabetic subjects in this

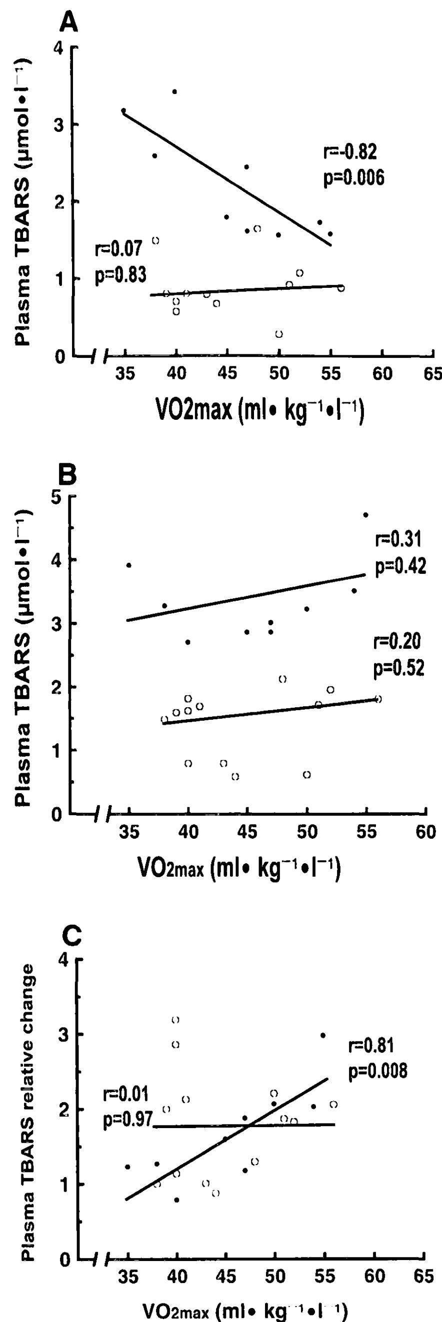

Figure 2—*Pre-exercise (A) and postexercise (B) plasma TBARS levels, and the relative change of plasma TBARS (C) (postexercise TBARS/pre-exercise TBARS) as a function of maximal oxygen consumption in the diabetic (●) and control (○) groups.*

study were in moderate glycemic control and had few complications. Our findings are consistent with other studies reporting elevated plasma TBARS or MDA, even in diabetic patients without complications (9,11–13). Correlations between plasma TBARS and glycemic control or lipid fractions have been found in diabetic patients (8), although others have had contradictory findings (9–11). We found no such correlations reaching statistical significance.

The diabetic group had a higher resting level of blood TGSH than the control subjects. The GSSG to TGSH ratio was similar in both groups. Higher blood TGSH levels in diabetes are in contrast to other human studies finding lower levels (21, 22). Experimental models of diabetes consistently show decreased levels of GSH in erythrocytes or tissues normally high in GSH (19,23–24). In a study also looking at blood GSSG levels, GSSG/TGSH was significantly elevated in diabetic patients (21). An increased GSSG to TGSH ratio has also been found in experimental diabetes (19). In animal models, decreased GSH corrects with insulin (23) or aldose reductase inhibitors (24). In contrast to our study, these studies support the hypothesis that in diabetes, hyperglycemia leads to activation of NADPH-dependent aldose reductase, which decreases the amount of NADPH available for glutathione reductase, the activity of which is also NADPH-dependent. Such an effect may lower reduced GSH and raise GSSG levels. In the resting state, these changes have generally been interpreted as an impairment of antioxidant status in diabetes, predisposing to oxidative stress.

Of the above mentioned studies with human subjects, one dealt with older predominantly NIDDM patients (21). In the study by Jain and McVie (22), the patients were all children or adolescents with IDDM, and no mention was made of diabetic complications. Interestingly, a highly significant inverse correlation between blood TGSH and HbA_{1c} ($r = -0.59$) was reported in their study. We found a nearly identical correlation ($r = -0.58$), but possibly because of the small number of subjects, it did not reach statistical significance ($P = 0.10$). Furthermore, diabetic subjects in this study were generally physically active with normal Vo_{2max} values. Thus, the higher blood glutathione levels found in the diabetic group in the present study may be in part due to the clinical characteristics of the diabetic population in our study, which differed markedly from those in previous studies. Because of the complex interrelations and diverse functions of the various physiological antioxidants and antioxidant enzymes, far-reaching conclusions about antioxidant defenses should not be drawn from glutathione levels alone.

Diabetic and control groups showed a similar, ~50% increase in plasma TBARS from 40 min sustained exercise at 60% Vo_{2max}, although absolute postexercise plasma TBARS were remarkably elevated in diabetic versus control subjects. Increased plasma TBARS in response to exercise is in agreement with many studies examining sustained exercise-induced oxidative stress (25,33,39–41). Although free fatty acid or triglyceride levels were not measured immediately before or after exercise, net plasma triglyceride and free fatty acid levels of fatty acids containing two double bonds (the minimum for significant TBARS formation) or more remained virtually unchanged in response to 30 and 60 min of variable intensity exercise (42). Therefore, it is unlikely that lipid changes during exercise are a significant cause of exercise induced TBARS formation.

The dramatic increase in blood GSSG and GSSG/TGSH in response to exercise in this study also indicates increased oxidative stress resulting from exercise, although there were no significant differences in these indexes between diabetic and control groups. Acute changes in glutathione redox status have been considered to be a particularly sensitive indicator of oxidative stress (25,32,33, 43,44). This response of GSSG and GSSG/TGSH to acute exercise is also in agreement with previous studies by us (25, 32,33) and others (43,44). We have found no other studies published assessing exercise-induced oxidative stress in diabetes.

The rise in plasma TBARS relative to resting values correlated directly with oxygen consumption during exercise (at 60% Vo_{2max}). Vo_{2max} did not correlate with absolute postexercise TBARS. This, coupled with the finding that plasma TBARS at rest was inversely correlated with Vo_{2max}, suggests that although exercise-induced oxidative stress in diabetic individuals increases with increasing oxygen consumption, it is partially offset (a relative rather than absolute increase in TBARS) by good physical fitness and lower resting levels of oxidative stress. Although it may at first seem paradoxical that more fit IDDM subjects had greater relative increases in plasma TBARS, the more fit IDDM subjects consumed up to

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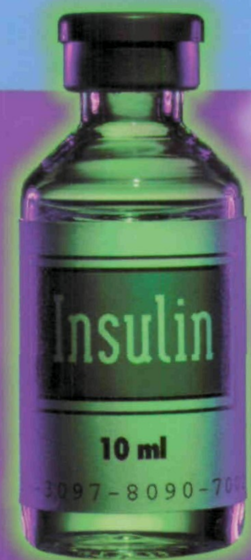

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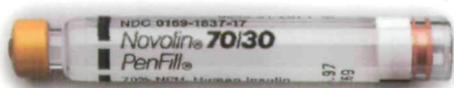

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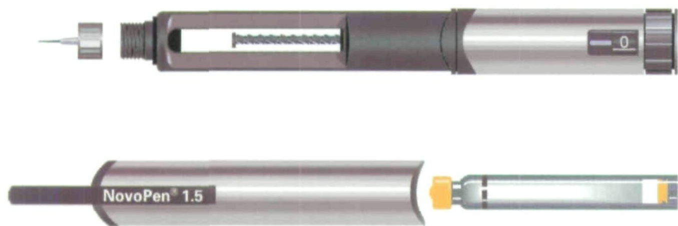

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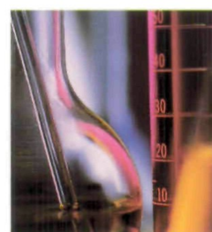

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1.6 times as much oxygen during exercise than the less fit, subjecting them to greater pro-oxidant forces. Of side interest is the wide variability of the relative TBARS increase seen in the less fit control subjects, which may indicate more heterogeneity in antioxidant defenses or in susceptibility to oxidative stress. The fact that the correlations between Vo_{2max}, resting plasma TBARS, and relative rise with exercise were not present in the control group in the present study nor reported in previous studies (33,40–41) may be because of the variability in the less fit control subjects, their much lower resting levels of oxidative stress, and because fitness may play a more central role as a determinant of resting oxidative stress in diabetes. This could be of key importance because of increasing evidence implicating oxidative stress in the accelerated atherosclerosis and microvascular complications of diabetes (2,4–5).

Vo_{2max} has also been found in healthy young men to correlate with vastus lateralis muscle activities of superoxide dismutase and catalase, major endogenous antioxidant enzymes (45). The relationship between oxidative stress and antioxidant defenses is complex. Upregulation of antioxidant defenses can occur in response to increased oxidative stress or result in decreased oxidative stress (29–30). Exercise training in nondiabetic animals has been shown, however, to reduce indexes of lipid peroxidation in heart (46) and skeletal muscle (39). Training has also been shown to have favorable effects on oxidative stress and antioxidant status as measured by TGSH and GSSG (32,46–47) and glutathione peroxidase, catalase, and superoxide dismutase activities (32,48). Increased activity of antioxidant enzymes has been reported in muscles with high oxidative capacity (32,39,48). Training has been most beneficial in these same muscles (32,39,48). Regular physical activity and higher Vo_{2max} may thus lower oxidative stress by strengthening antioxidant defenses and permitting more efficient use of oxygen. Our study would imply that regular exercise or training would also have a protective effect on oxidative stress in diabetic patients. Clarification of the role of fitness and training in diabetic oxidative stress would require a longitudinal study, which is currently underway.

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